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Histological Studies of Post-Mortem Changes in Sarcomere Length as Related to Bovine Muscle Tenderness

SUMMARY

The effect of post-mortem muscle contraction on ultimate tenderness was studied in muscles of 12 beef animals of similar weight and grade. State of contraction was determined by measurement of sarcomere lengths. Longissimus dorsi and semimembranosus muscles were observed. Histological samples removed at various intervals post-mortem were treated with ethylenediaminetetraacetate (EDTA) to prevent further contraction. A phase-contrast microscope was used to observe muscle fibers, and sarcomeres were measured with a filar micrometer. Muscle contraction patterns of each animal were plotted through a 7-day aging period. State of contraction after 7 days appeared to have a greater influence on subsequent (7 days) tenderness than did state of contraction at time of maximum rigor mortis. Although contraction did not seem to be the factor most responsible for final tenderness, it did appear to have a significant influence. Considerable lengthening of sarcomeres normally occurred during the aging period. Semimembranosus muscles routinely contracted less than longissimus dorsi muscles during rigor mortis, and were more relaxed after 7 days of aging. Semimembranosus muscles were consistently less tender than longissimus dorsi muscles at slaughter, but the reverse was usually true after 7 days.

During recent years, considerable interest has been focused on relationships between post-mortem muscle contraction and tenderness. Locker (1960) observed that relaxed muscles were more tender than partially contracted muscles 2 days post-mortem. This was more evident in muscles low in connective-tissue content. Partmann (1963) impeded post-mortem actomyosin formation in strips of a diaphragm muscle by allowing them to age in a weighted, stretched condition. The weighted strips were significantly more tender than the unweighted controls. It was earlier suggested that increased tenderness during aging may be related to dissociation of actomyosin (Wierbicki *et al.*, 1956). However, there has been no conclusive proof that such a dissociation actually occurs. Wierbicki *et al.* (1956) indicated that although actomyosin appeared to play a role in meat tenderness, no evidence of actomyosin dissociation during aging was found. Similar conclusions have been reached by Neelin and Rose (1964) with chicken muscle. Yet, fiber fragments of aged muscle were shown to contract upon addition of ATP, suggesting at least some dissociation during aging (Partmann, 1963).

If sarcomere length decreases with an increase in actomyosin, or vice versa, it would appear logical that measurements of sarcomere lengths at various intervals post-mortem would reflect the degree of actomyosin dissociation during the aging period. Therefore, measurements have been made to determine the extent of maximum muscle contraction at rigor mortis, and the subsequent amount of relaxation occurring during a 7-day aging period. The relationships of these changes in bovine muscles to ultimate 7-day tenderness values were investigated.

METHODS

Selection and treatment of carcasses. Carcasses from 4 bulls, 4 heifers, and 4 steers were used. The animals were of various beef breeds but were similar in weight and grade. Carcasses weighed 500–600 lb, and graded high-good to low-choice.

In order to create different contraction patterns between the two sides of each carcass, different initial holding temperatures were employed. Immediately after dressing, the right side of each carcass was subjected to 1–2°C for rapid chilling. The left side was left at 20°C for 9 hr, and then placed with the other side. After 48 hr, both sides were transferred to a holding cooler and maintained at 3°C throughout the remainder of a 7-day aging period.

Sampling methods. Longissimus dorsi and semimembranosus muscles were sampled from each side of every carcass. Two types of samples were taken: shear samples for tenderness evaluations, and histological samples for microscopic observations.

Steaks 3.17 cm thick were excised both immediately after stunning and after 7 days' aging. In bulls, initial samples were taken approximately 1 hr after death, but heifer and steer samples were removed within 5 min of stunning. Initial longissimus dorsi samples were removed from over the 13th rib of the left side. Samples from semimembranosus muscles were taken parallel to the ischium. Corresponding muscles of the right side were also severed in order to create the same effect of cutting. All steaks were in the cooker within 10 min of removal and were cooked in deep fat at 135°C to an internal temperature of 70°C. Following a 5-min cooling period at room temperature, three 2.54-cm cores were removed from each steak and sheared twice with a Warner-Bratzler shear machine. The average of the 6 readings was used for shear value.

Samples were removed for histological examination at various intervals post-mortem. Beginning at the time of slaughter, a sample from each muscle was taken every 3 hr for the first 24 hr. Two samples were then taken at 6-hr intervals, and one was taken each day throughout the remainder of the aging period. All samples were immediately frozen in OCT compound. Frozen tissue blocks were sectioned with a microtome cryostat, and sections were collected onto slides treated with EDTA to prevent thaw contraction. Slides were observed with a phase-contrast microscope, and sarcomeres were measured with a filar micrometer. Five muscle fibers were selected at random from each section, and 5 consecutive sarcomeres were measured in each fiber. The average of 25 readings was used as sarcomere length of each muscle at any given time.

RESULTS AND DISCUSSION

Extreme contraction was noted in prerigor muscle samples when sections were collected onto clean untreated slides. Such thaw rigor has been reported to occur from calcium release into the actomyosin system (Partmann, 1961). Since it was necessary to observe muscle fibers in exactly the state of contraction as when removed from the carcass, a method was devised to prevent thaw contraction. By employing EDTA to chelate calcium ions, state of contraction of a selected muscle at time of sampling could readily be observed and measured. When frozen sections were collected onto slides treated with glycerine and saline solution saturated with EDTA, further contraction did not occur. Fig. 1A shows muscle fibers not treated with EDTA. In this case, fibers

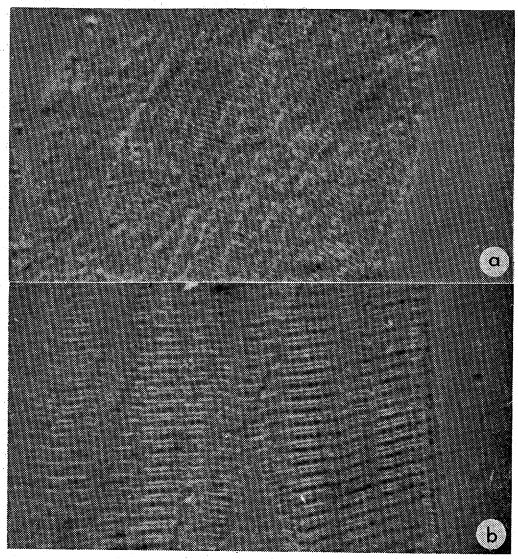

Fig. 1. A) Muscle fibers excised prior to onset of rigor mortis. The frozen section was collected onto a clean slide, and extreme thaw contraction occurred. B) An adjacent section from the same sample as *A*, but collected onto a slide treated with EDTA. Thaw contraction was prevented, as evidenced by more relaxed fibers.

contracted greatly upon thawing. Fig. 1B shows EDTA-treated fibers from an adjacent section of the same sample in which fibers were not in a contracted state.

By this procedure it was possible to observe fiber changes and to plot muscle contraction patterns of each animal. Many samples excised soon after stunning contracted greatly upon cutting, which corresponds to previous findings (Locker, 1960; Herring *et al.*, 1965). This was especially true in one heifer that showed evidence of nervousness immediately previous to slaughter. However, as rigor mortis approached, muscles lost this irritability and very little response to cutting occurred after 3 hr post-mortem.

There was no significant difference in sarcomere lengths of longissimus dorsi and semimembranosus muscles during the delay period. These values were consistently about 2 μ. Observed changes suggested that cut muscles did not recover to their original lengths prior to cutting, for considerably longer sarcomeres have been reported for psoas major (Locker, 1960) and semitendinosus muscles (Herring *et al.*, 1965).

As the rapid phase of contraction began,

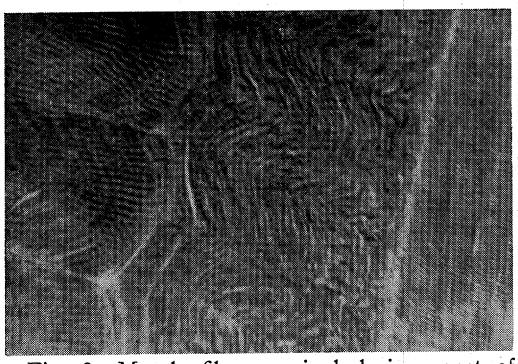

Fig. 2. Muscle fibers excised during onset of rigor mortis. The most contracted fiber, at right, is straight, whereas the less contracted ones are wavy.

muscle fibers contracted at various rates. Rapidly contracting fibers were normally straight in appearance, while adjacent slower contracting fibers were usually wavy or "kinked" in appearance. There was evidence that kinking was due to a pulling influence of rapidly contracting fibers on those more relaxed, as shown in Fig. 2.

The two muscle types observed revealed somewhat different contraction patterns. Semimembranosus muscles normally did not reach the state of contraction of that observed in the longissimus dorsi muscles during rigor mortis (Table 1).

During rigor, semimembranosus muscles contracted to an average of 78.4% of prerigor values, while longissimus dorsi muscles were contracted to 73.7%. Muscles from sides initially exposed to the 20°C treatment usually showed greater contraction than those held at 1–2°C. Correlation coefficients (Table 2) indicated only a small relationship between state of contraction at maximum rigor mortis and the ultimate (7 days) tenderness.

Yet, within animals, more contracted muscles of the two treatments usually proved to be ultimately less tender. In 16 comparisons with heifers and steers, muscles reaching greater contraction during rigor mortis were ultimately less tender in 12 instances. This difference proved significant ($P < .05$) with semimembranosus muscles.

Table 1. Average sarcomere lengths (μ) of longissimus dorsi and semimembranosus muscles during maximum contraction.

	Longissimus dorsi		Semimembranosus	
	1–2°C	20°C	1–2°C	20°C
Bulls	1.34	1.52	1.70	1.67
Heifers	1.55	1.45	1.68	1.51
Steers	1.54	1.54	1.60	1.55
Av.	1.50	1.48	1.66	1.57
	1.49		1.62	

Table 2. Correlation coefficients of maximum contraction during rigor mortis and ultimate tenderness.

	Longissimus dorsi	Semimembranosus
Bulls	—.57	.05
Heifers	—.32	—.56
Steers	—.18	.16
Heifers and steers	—.47*	—.23
Heifers, steers, and bulls	—.38	—.11

* Significant at .05 level.

Normally, sarcomere lengths began to increase less than 3 hr after maximum contraction had been reached. This was most marked in the semimembranosus muscles, which were in most cases considerably more relaxed after 7 days than longissimus dorsi muscles. By the 7th day, values for semimembranosus muscles were 90.4% those of prerigor, and values for longissimus dorsi muscles averaged 86.5%.

Semimembranosus muscles were consistently less tender than longissimus dorsi muscles immediately after slaughter (Table 3). Average initial shear values were 18.62 kg and 12.15 kg, respectively. However, these tenderness trends were normally reversed after 7 days. Average 7-day shear value was 10.3 kg for semimembranosus muscles, and 12.18 kg for longissimus dorsi muscles. Semimembranosus muscles underwent considerable tenderization during aging, but final longissimus dorsi shear values differed very little from initial values. In three of the four heifer carcasses, longissimus dorsi muscles were considerably less tender after 7 days.

Final state of contraction appeared to be closely associated with tenderness. After 7 days' aging, more contracted longissimus dorsi muscles, between the two treatments, were significantly ($P < .05$) less tender than less contracted muscles. This was also true of semimembranosus muscles from heifers and steers.

Table 3. Initial and final shear values [a] of two muscles (in kg).

Animal	Treatment [b]	Longissimus dorsi		Semimembranosus	
		Initial shear	Final shear	Initial shear	Final shear
Bull 1	R	16.11	17.10	21.05	15.62
	C		13.88		12.02
Bull 2	R	14.40	9.01	24.29	9.61
	C		9.95		9.52
Bull 3	R	11.71	12.66	18.20	8.75
	C		13.85		7.36
Bull 4	R	13.65	12.21	14.67	11.72
	C		13.52		6.93
Heifer 1	R	11.81	12.69	18.55	11.25
	C		12.61		12.08
Heifer 2	R	13.55	12.19	17.91	9.88
	C		7.74		8.21
Heifer 3	R	9.37	12.74	19.56	10.58
	C		12.04		9.13
Heifer 4	R	10.66	21.86	20.09	17.04
	C		19.73		14.88
Steer 1	R	10.45	9.71	13.57	8.48
	C		13.73		9.39
Steer 2	R	9.77	8.20	14.86	9.61
	C		8.84		7.08
Steer 3	R	11.47	11.06	17.40	9.73
	C		9.92		10.28
Steer 4	R	12.99	9.71	23.40	8.31
	C		8.20		7.44

[a] Shear value represents mean value of six shear determinations.
[b] R = initial treatment of 20°C; C = initial treatment of 1–2°C.

It was often very difficult to section samples removed after 6 or 7 days of aging. The fibers appeared to be brittle, and were easily shattered. Shattering always appeared to occur at the levels of the I bands, which suggested occurrence of some type of degradation of the protein actin during aging. Such a degradation could possibly be associated with tenderization during the aging period. The degree of contraction after 7 days aging may be a significant factor influencing muscle tenderness, particularly in muscles where contraction occurs rather uninhibited as in the longissimus dorsi.

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